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(71) Applicant *(for all designated States except US)*: **WAYNE STATE UNIVERSITY** [US/US]; 656 West Kirby, Detroit, MI 48202 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **TERLECKY, Stanley, R.** [US/US]; 739 Westview Road, Bloomfield Hills, MI 48304 (US). **WALTON, Paul, A.** [CA/CA]; 466 Billybrook Crescent, London, Ontario N5X 2Y8 (CA).

(74) Agent: **LIVNAT, Shmuel**; McKenna Long & Aldridge LLP, 1900 K Street, N.W., Washington, DC 2006 (US).

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(54) Title: PROMOTION OF PEROXISOMAL CATALASE FUNCTION IN CELLS

(57) Abstract: The molecular mechanisms of peroxisome biogenesis have begun to emerge; in contrast, relatively little is known about how the organelle functions as cells age. The present inventors characterized age-related changes in peroxisomes of human cells and showed that aging compromises peroxisomal targeting signal 1 (PTS1) protein import, with the critical antioxidant enzyme, catalase, especially affected. The number and appearance of peroxisomes are altered in these cells, and the organelles accumulate the PTS1-import receptor, Pex5p, on their membranes. Concomitantly, cells produce increasing amounts of the toxic metabolite, H_2O_2, and this increased load of reactive oxygen species (ROS) may further reduce peroxisomal protein import and exacerbate the effects of aging. Disclosed are novel compositions and methods for restoring catalase in peroxisomes by use of targeted catalase modified at its C-terminus and/or N-terminus, optionally in combination with polypeptides which promote cellular uptake of proteins, to prevent or overcome the changes that follows aging or that are associated with a number of diseases or disorders.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/34512

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12N 9/08, 15/53; A61K 38/44
US CL : 435/192; 536/23.2; 424/94.3, 94.4
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/192; 536/23.2; 424/94.3, 94.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	SHEIKH, F.G. et al. Abnormality in Catalase Import Into Peroxisomes Leads to Severe Neurological Disorder. Proc. Natl. Acad. Sci. March 1998, Vol. 95, pages 2961-2966, see entire document.	1-8, 12, 35, 38 ---------- 20, 21, 23, 24, 26, 27, 29, 30, 32-34, 36, 37, 39-47, 49, 50, 52-55
X --- Y	MIURA, S. et al. Carboxy-Terminal Consensus Ser-Lys-Leu-Related Tripeptide of Peroxisomal Proteins Functions in Vitro as a Minimal Peroxisome-Targeting Signal. J. Biol. Chem. 15 July 1992. Vol. 267, No. 20, pages 14405-14411, see particularly paragraph bridging pages 14407 and 14408.	1-8,12,35,38 ---------- 20,21,23,24,26,27,29, 30,32-34,36,37,39-47,49,50,52-55
Y	FUJIWARA, C. et al. Catalase-less Peroxisomes. J. Biol. Chem. 24 November 2000, Vol. 275, No. 47, pages 37271-37277, see particularly pages 37275-37276.	1-12,20,21,23,24,26,27, 29,30,32-38,41,42,45-47,49,50,53

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 March 2005 (28.03.2005)	19 JUL 2005
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner of Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Rebecca E. Prouty Telephone No. 571-272-1600

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/34512

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 1-12,20,21,23,24,26,27,29,30,32-47,49,50,52-55 as related to PTS1

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☒ No protest accompanied the payment of additional search fees.

PCT/US03/34512

INTERNATIONAL SEARCH REPORT

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	TRELEASE, R.N. et al. Rat Liver Catalase is Sorted to Peroxisomes By Its C-Terminal Tripepeptide Ala-Asn-Leu Not By the Internal Ser-Lys-Leu Motif. Eur. J. Cell Biol. November 1996. Vol. 71, pages 248-258, see particularly Table II.	1-11,35,38 ---------- 12,20,21,23,24,26,27,29,30,32-34,36,37,39-47,49,50,52-55
Y	MORRIS, M.C. et al. A Peptide Carrier For the Delivery of Biologically Active Proteins Into Mammalian Cells. Nature Biotecnology. Dec. 2001. Vol. 19, pages 1173-1176, see entire document.	23,24,26,27,29,30,32,36,41-44
E	US 2004/0058856 A1 (CHOI et al.) 25 March 2004 (25.03.2004), see entire document.	20,2123,24,26,27,29,30,35-47,49,50,52-55

INTERNATIONAL SEARCH REPORT

PCT/US03/34512

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-12, 20, 21, 35, and 37-40, drawn to a modified catalase having a carboxy-terminal PTS1 sequence (i.e., S/A/C-K/R/H-L/M), compositions thereof and use thereof for reducing the concentration of hydrogen peroxide in a cell.

Group II, claim(s) 13, 14, 22, 35, and 37-40, drawn to a modified catalase having a PTS2 sequence (i.e., K/R -L/I/V-X_5-H/Q-A/L/F) at or near the amino terminus, compositions thereof and use thereof for reducing the concentration of hydrogen peroxide in a cell.

Group III, claim(s) 15, 16, 22, 35, and 37-40, drawn to a modified catalase having a PTS2 sequence (i.e., K/R -L/I/V-X_5-H/Q-A/L/F) at or near the amino terminus and a carboxy-terminal PTS1 sequence (i.e., S/A/C-K/R/H-L/M), compositions thereof and use thereof for reducing the concentration of hydrogen peroxide in a cell.

Group IV, claim(s) 17-19, drawn to nucleic acids or host cells encoding a modified catalase having a carboxy-terminal PTS1 sequence (i.e., S/A/C-K/R/H-L/M), and use thereof for producing the modified catalase.

Group V, claim(s) 17-19, drawn to nucleic acids or host cells encoding a modified catalase having a PTS2 sequence (i.e., K/R -L/I/V-X_5-H/Q-A/L/F) at or near the amino terminus, and use thereof for producing the modified catalase.

Group VI, claim(s) 17-19, drawn to nucleic acids or host cells encoding a modified catalase having a PTS2 sequence (i.e., K/R -L/I/V-X_5-H/Q-A/L/F) at or near the amino terminus and a carboxy-terminal PTS1 sequence (i.e., S/A/C-K/R/H-L/M), and use thereof for producing the modified catalase.

Group VII, claim(s) 23, 24, 26, 27, 29, 30, 32-34, 36, and 41-44, drawn to a peroxisomally-targeted polypeptide comprising a modified catalase having a carboxy-terminal PTS1 sequence (i.e., S/A/C-K/R/H-L/M) and a delivery or translocation molecule or moiety bound thereto and use thereof for reducing the concentration of hydrogen peroxide in a cell.

Group VIII, claim(s) 25, 28, 31, 36, and 41-44, drawn to a peroxisomally-targeted polypeptide comprising a modified catalase having a having a PTS2 sequence (i.e., K/R -L/I/V-X_5-H/Q-A/L/F) at or near the amino terminus and a delivery or translocation molecule or moiety bound thereto and use thereof for reducing the concentration of hydrogen peroxide in a cell.

Group IX, claim(s) 25, 28, 31, 36, and 41-44, drawn to a peroxisomally-targeted polypeptide comprising a modified catalase having a having a PTS2 sequence (i.e., K/R -L/I/V-X_5-H/Q-A/L/F) at or near the amino terminus and a carboxy-terminal PTS1 sequence (i.e., S/A/C-K/R/H-L/M) and a delivery or translocation molecule or moiety bound thereto and use thereof for reducing the concentration of hydrogen peroxide in a cell.

Group X, claim(s) 45-47, 49, 50, and 52-55, drawn to methods of treating a disease associated with inadequate levels of peroxisomal catalase using a modified catalase having a carboxy-terminal PTS1 sequence (i.e., S/A/C-K/R/H-L/M).

Group XI, claim(s) 45, 48, 49, 50, and 52-55, drawn to methods of treating a disease associated with inadequate levels of peroxisomal catalase using a modified catalase having a PTS2 sequence (i.e., K/R -L/I/V-X_5-H/Q-A/L/F) at or near the amino terminus.

Group XII, claim(s) 45, 48, 49, 50, and 52-55, drawn to methods of treating a disease associated with inadequate levels of peroxisomal catalase using a modified catalase having a PTS2 sequence (i.e., K/R -L/I/V-X_5-H/Q-A/L/F) at or near the amino terminus and a carboxy-terminal PTS1 sequence (i.e., S/A/C-K/R/H-L/M).

Group XIII, claim(s) 51, drawn to methods of treating or preventing skin wrinkling using a modified catalase having a carboxy-terminal PTS1 sequence (i.e., S/A/C-K/R/H-L/M).

Group XIV, claim(s) 51, drawn to methods of treating or preventing skin wrinkling using a modified catalase having a PTS2 sequence (i.e., K/R -L/I/V-X_5-H/Q-A/L/F) at or near the amino terminus.

Group XV, claim(s) 51, drawn to methods of treating or preventing skin wrinkling using a modified catalase having a PTS2 sequence (i.e., K/R -L/I/V-X_5-H/Q-A/L/F) at or near the amino terminus and a carboxy-terminal PTS1 sequence (i.e., S/A/C-K/R/H-L/M).

The inventions listed as Groups I-XV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the only shared technical feature of Groups I-XV is that each relate to a modified catalase comprising one or more non-natural peroxisome targeting sequences. However, this shared technical feature is not a special technical feature as defined by PCT Rule 13.2 as it does not constitute a contribution over the art. Sheik et al. (PNAS 95 : 2961, 1998) teach modified catalases comprising a carboxy terminal PTS1 sequence (i.e., SKL) or a PTS2 sequence (i.e., HRLQVVLGHL) immediately following the initial methionine. As such neither the modified catalase fusions nor the PTS1 or PTS2 peptides themselves are special technical features. Furthermore, Groups X-XII or XIII-XV do not have unity with Groups I-III as Groups I-III already include a method of use of the modified catalaise which comprises unrelated steps to the methods of Groups X-XII or XIII-XV and 37 CFR 1.475 does not provide for the inclusion of multiple methods of use within the main invention.

Continuation of B. FIELDS SEARCHED Item 3:
EAST, MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, CA, NTIS, ESBIOBASE, BIOTECHNO, WPI search terms: catalase#, peroxisom?, target? or import? or transport?, treat? or pharmaceutical#, human